Biological Applications of medicinally Valued *Punica granitum*

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Abstract The research study aimed to evaluate the pharmacological activities of Punica granatum (pomegranate) extract, specifically focusing on its analgesic, anti-inflammatory, and anti-spasmodic properties. The crude extract of Punica granatum was prepared using different solvents, namely aqueous and methanol. The presence or absence of chemical constituents in the extracts was tested. The study found that the aqueous extract of Punica granatum, administered at a dose of 300 mg/kg, exhibited a significant analgesic effect (12.0±3.45), which was higher than the effect of the standard drug aspirin (5.0±1.71). On the other hand, the methanol extract of Punica granatum, also administered at a dose of 300 mg/kg, demonstrated a notable anti-inflammatory effect (0.60±0.33) after four hours, effectively reducing inflammation compared to the standard drug diclofenac sodium (1.26±1.11) after the same time period. Moreover, the methanol extract of Punica granatum, at a dose of 300 mg/kg, showed a favorable effect on the total length of the intestine (60.0±3.45) and the length of the charcoal meal (13.0±6.24) compared to the effect of the standard drug atropine sulfate, which had a total intestine length of 58.6±3.6 and a charcoal meal length of 6.6±3.6. These results indicate that both the methanol and aqueous extracts of Punica granatum possess analgesic, anti-inflammatory, and anti-spasmodic properties, suggesting that further detailed studies should be conducted.

Index Term pharmacology Analgesic, Antispasmodic, Anti-inflammatory, Metabolite

I. INTRODUCTION

Pharmacology is the branch of science that focuses on the study of drugs and their effects on the body. It encompasses various aspects of drugs, including their sources, chemical characteristics, mechanisms of action, therapeutic uses, adverse effects, and interactions with biological systems. (Bahrami *et al*, .2011). Pharmacology, as a broad field, encompasses the study of drugs and their effects on the body. It involves understanding how drugs interact with biological systems, including their mechanisms of action, therapeutic uses, and potential side effects. Pharmacologists are primarily interested in the therapeutic and toxicological aspects of drugs and chemical agents. However, it's worth noting that pharmacology as a whole extends beyond these two categories, covering various other sub disciplines and research areas. (Ncube *et al*,.2008). Pharmacokinetics refers to the processes by which a drug is absorbed, distributed, metabolized, and eliminated by the body, while pharmacodynamics refers to the biochemical and

Physiological effects of drugs on the body and the mechanisms of their action. In drug development, understanding how drugs interact with cellular targets, typically through binding to specific receptors, is crucial. This knowledge allows pharmacologists to design drugs that are more selective in their action and have fewer undesirable side effects. By focusing on the steps involved in drug-receptor interactions, researchers can develop drugs that modulate these processes effectively. Additionally, you mentioned that plants with a long history of use in medicine can serve as a rich source of substances for treating various disorders and infectious diseases. This statement is correct. Many pharmaceutical compounds have been derived from natural sources, including plants, and traditional medicine practices have often utilized plant-based remedies. The study of these medicinal plants can lead to the discovery of new bioactive compounds that may have therapeutic potential for the treatment of various ailments.

(Fransworth *et al*,. 1985). Phytochemicals are bioactive compounds that are naturally present in plants. They are responsible for the vibrant colors, flavors, and aromas found in fruits, vegetables, herbs, and other plant-based foods. These compounds serve various functions in plants, such as protecting them from environmental stressors, pests, and diseases. (Hasler and Blumberg1999). These chemicals protect from different diseases and its cells from environmental hazards (Gibson and wardel *et al.*,1998)(Mathai 2000).There are more than 4000 phytochemicals identified but only 150 are studied in detail (American Cancer Society 2000) Phytochemicals are classified primary constituents such as proteins, amino acids, sugar and secondary constituent such as terpens, steroids, phenolics (hanh 1998)Phytochemicals are present and accumulates in different parts of plants such as fruits, vegetables, seeds and sometimes in fungi etc (Costa and Zia *et al.*,1999).Phytochemicals also found in supplement form but still it is not sure that either the great role as dietary phytochemical (American Cancer Society 2000).Phytochemicals are produce in plants in metabolic processes so called secondary metabolites having many biological roles namely detoxification Activity, enhancing immunity, and anticancer property. It is also known that these substances are produce by plants for their protection, but recent studies shows that some phytochemicals protect human against illness (Narsinga, 2003) The study of those chemicals produce by plants produce as a secondary metabolites i-e for self-defense. It deals with the synthesis, composition, structure and role of phytochemical in health and other industrial processes. Discovery of drugs and development of new therapeutic agents against major diseases depend upon efficient understanding of phytochemicals (Saxena *et al.,* 2013) Natural products, mainly those obtain from plants, have been used to help mankind maintain health since the dawn of medicine. Medicinal plants were used as a source for curing in local communities in all over the world for thousands of years. Still it did not loss his significance and use as a primary

healthcare form for approximately 85% of the world's population and more than 80% drugs are still synthesized from plants (Bauer and Bronstrup, 2004). Human were searching for such drugs which have positive effect on health with minute side effects which were found in plants products which attract attention of scientists in all over the world. In the pharmaceutical site, plants are rich source of those substances which are used for the treatment of various disorders and other infections. Therapeutic effects of medicinal plants include antiviral, anticancer, antimalarial, and analgesic properties. According to WHO most of the drugs obtained from plants and about 80% of the world's population depends on these traditional medicines for their primary health care needs (Fransworth, *et al.*, 1985). *Punica grana tam* is commonly known as Anar. It belong to family Punicaceae. It is a small tree found mostly in Asia (Das and Mandal *et al.*, 1999)

II METRIAL AND METHODS

Plant Collection and preservation

The extraction of compounds from Punica granatum (pomegranate) peel using water and methanol as solvents. Punica granatum peel was collected from Swabi, Khyber Pakhtunkhwa Province. The plant was taxonomically identified by Dr. Gul Jan, who is an Assistant Professor in the Department of Botany at Abdul Wali Khan University Mardan (AWKUM). The collected plant peel was washed with tap water to remove any contaminants. After that, it was dried at room temperature. Once dried, the plant material was ground into a powder using a grinder and stored in an airtight bottle. Specific amounts of this powder were weighed for further processing. Two separate solutions were prepared using the plant powder. One solution involved dissolving 30 grams of the powder in 400 ml of distilled water, while the other solution involved dissolving another 30 grams of the powder in 400 ml of methanol. These two solutions were placed in separate conical flasks and shaken in a shaker for 72 hours. After 72 hours of shaking, both solutions were filtered through Whatman filter paper in separate rotatory bottles. This filtration process helps remove any solid particles or plant debris, leaving behind the extracted compounds in the liquid. The bottles were then labeled for identification. The filtered solutions were then placed in a water bath at 45°C for ten days. During this time, the water and methanol solvents evaporated, leaving a residue known as the crude layer at the bottom of each bottle. The study likely aimed to extract bioactive compounds present in the pomegranate peel using water and methanol as solvents. These compounds can have potential pharmacological or pharmacognostic applications and may be further analyzed and studied for their medicinal properties. However, additional information would be needed to understand the specific objectives and purpose of this study and the intended use of the crude layers obtained from the extraction process.(Van Wagenen *et al.* 1993).

Pharmacological activities

All activities related to pharmacology were carried out such as analgesic, anti-inflammatory and anti-spasmodic.

Experimental animals

Juvenile, healthy and 1-2 month of age mice were selected for this study. The animals were procured from veterinary research Centre Peshawar and were kept in their cages under hygienic conditions at AWKUM, Mardan and the temperature was maintained in the range of 25-30C^0. Equal length of light and dark cycle was provided to the animals for 12 hours each. All the mice were fed by food and water.

Analgesic Activity

Acetic acid induced writhing test was performed for analgesic activity. 1 ml of acetic acid was injected to all mice. After ten minutes of injection writhing were counted for 5 minutes then 1 ml of peel extract of water was injected in some mice and methanol extract to other. Then writhing was again measured for 5 minutes (Koster R *et al* .,1959).

Anti-inflammatory activity by carrageenan induced inflammation

Carrageenan induce paw edema test model was used for Anti-Inflammatory activities First diameter of paw was measured then 1 ml carrageenan was injected to all mice which cause inflammation in paw which was measured again after 1 hours of inflammation water and methanol extracts of plant were injected to all mice respectively and diameter was again measured and were compared with previous results which showed best effect in inflammation (Asif M *et al.*,2009).

Anti-inflammatory activity (%) = (1-D/C) X100,

Where, C= Mean paw volume of control, D= Mean paw volume of Test

Anti-spasmodic activity

Antispasmodic activity was performed by Charcoal meal test model .Charcoal was injected orally to mice after an hour the mice were dissected and the intestines were removed and stretched at paper and their length and distance travelled by charcoal was measured by measuring tape.

III. RESULT AND DISCUSSION

Analgesic activity

In the analgesic activity the writhing stimulated by acetic acid was carried out .the writhing values of control(G1) group of mice indicated 11.00±1.47 in per 5minutes the writhing value of (G2) indicated that 5.0±1.71with % inhibition show 58.33% the writhing (G3) methanol150mg/kg and (G4)methanol300mg/kg and(G5) aqueous 150mg/kg and(G6) aqueous 300mg/kg show that value(8.0±3.45),(14.0±3.45) (14.0±3.45.)(12.0±3.45 %) inhibition of (20%),(133.3%),(17.64%) and (40%) (Table 4.1, Figure 4.1)

Table 4.1 Analgesic activity of *Punica granatam*

Treatment	Dose	Groups	Initial Writhing	Final Writhing	%Inhibition
Control	1ML(saline)	G1	……	……	……
Standard	10mg/Kg	G2	12.0±1.71	5.0±1.71	58.33%
Methanol	150mg/Kg	G3	10.0±3.45	8.0±3.45	20%
Methanol	300mg/Kg	G4	6.0±3.45	14.0±3.45	133.3%
Aqueous	150mg/Kg	G5	17.0±3.45	14.0±3.45	17.6%
Aqueous	300mg/Kg	G6	20.0±3.45	12.0±3.45	40%

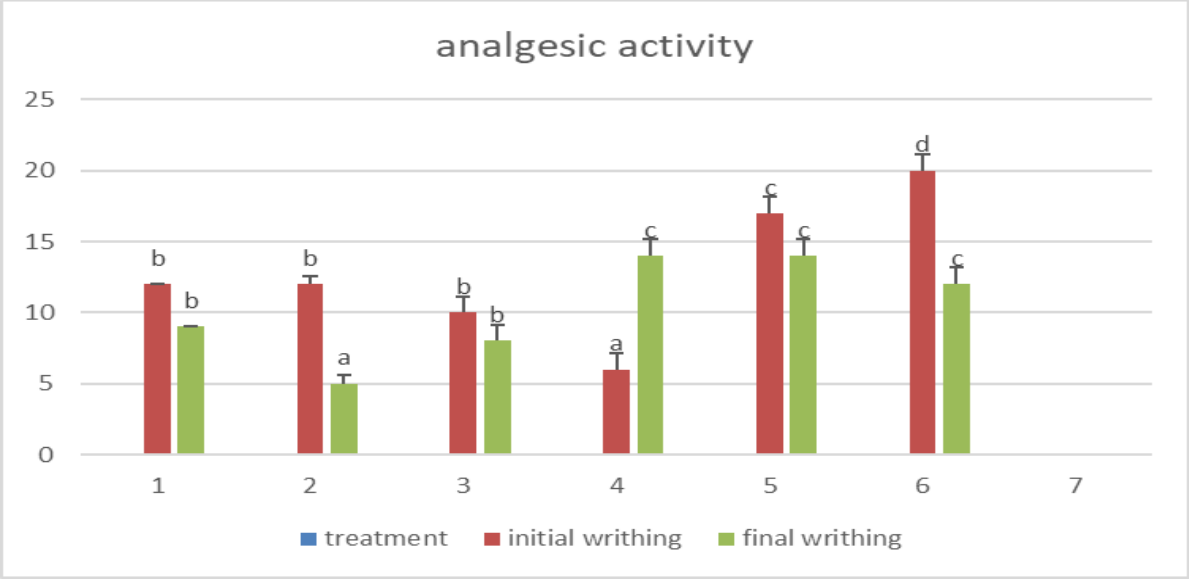


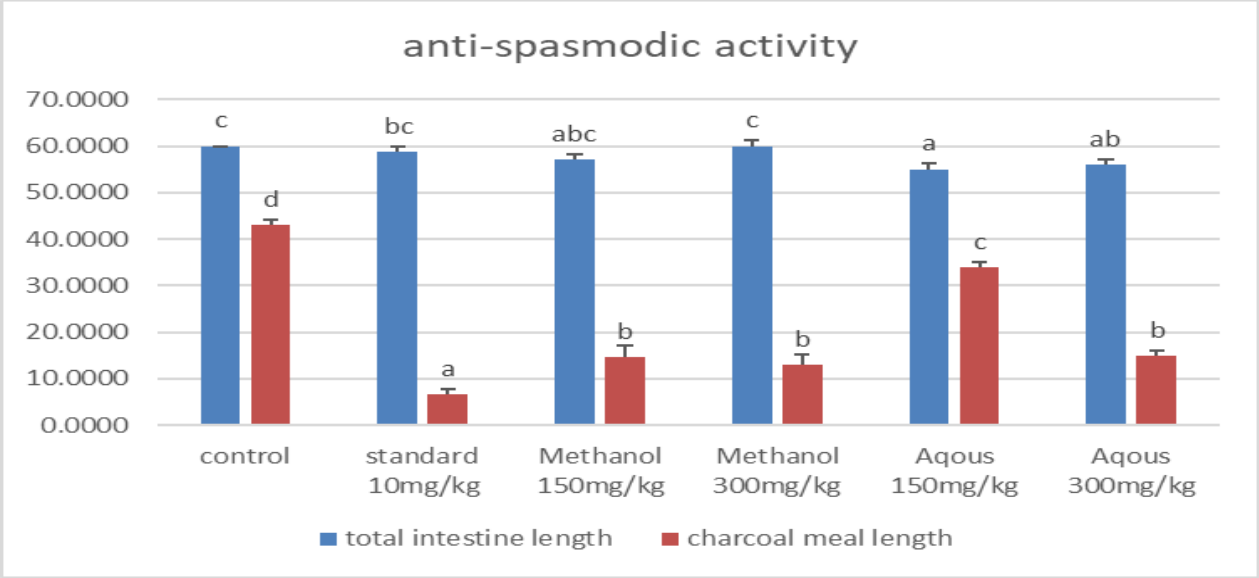
Figure 4.1 Comparison of Initial and Final Writhing

Anti-spasmodic Activity

In the present research work, the antispasmodic activity was carried out by charcoal. Control group showed 41.50 spasm value/5minutes. The spasm total intestinal length and charcoal meal length values atropine sulphate treated group G2 showed 6.6±3.6respectively .The mice of group 3rd with dose of 150mg/kg Methanol were scarified. The values for intestinal length of methanol 50mg/kg were measured as, 57.0±3.45 methanol 300mg/kg 60.0±3.45 aqueous150mg/kg55.0±3.45 aqeous 300mg/kg 56.0±3.45 for charcoal meal length revealed that methanol 150mg/kg, methanol mg/kg300 aqueous 150 and aqueous 300mg/kg(14.6±7.2),(13.0±6.24),(34.0±3.45),(15.0±3.45) respectively. The most efficient extrect was found aqueous 300mg/kg followed by aqueous 150mg/kg (Fig 4.2, Table 4.2)

Table 4.2 Showing Anti-Spasmodic activity Of *Punica granatum*

Treatment	Dose	Groups	Total intestine Length	Charcoal meal Length	% Inhibition
Normal		G1	….	….	…..
Standard	10mg/kg	G2	58.6±3.6	6.6±3.6	88.72%
Methanol extract	150mg/kg	G3	57.0±3.45	14.6±7.2	74.38%
Methanol extract	300mg/kg	G4	60.0±3.45	13.0±6.24	78.33%
Aqous extract	150mg/kg	G5	55.0±3.45	34.0±3.45	38.18%
Aqous extract	300mg/kg	G6	56.0±3.45	15.0±3.45	73.21%



4.2 Graphical representation Anti-spasmodic Activity

Anti-Inflammatory Activity:

Anti-inflammatory activity was conducted by Carrageenan induced paw edema. The volume of paw in mm for control group was G1 which was 1.53±0.33. Standard drug treated group is G2 1.16±0.33 before administration of carrageenan by the dose of 150mg/kg after 1h Methanol, aqueous, G3 0.80±0.33, 1.20±0.33. The inflammation values after 2hr of Carrageenan administration. Methanol and aqueous at a dose (300mg/kg) showed the value of 1.00±0.33, 1.10±0.33 respectively.

Table 4.3 Showing Anti-Inflammatory Activity of *Punica granatum*

Treatment	**Dose**	**Groups**	**1 hour**	**2 hour**	**3 hour**	**4 hour**
Control		G1	1.53±0.33	1.64±0.33	1.68±0.33	1.73±0.33
standard	10mg/kg	G2	1.16±0.33	1.08±0.33	.99±0.33	1.26±1.11
Methanol	150mg/kg	G3	.80±0.33	.70±0.33	.60±0.33	.60±0.33
Methanol	300mg/kg	G4	1.00±0.33	.80±0.33	.70±0.33	.60±0.33
Aqueous	150mg/kg	G5	1.20±0.33	1.00±0.33	.60±0.33	.70±0.33
Aqueous	300mg/kg	G6	1.10±0.33	.86±0.72	.70±0.33	.60±0.33

DISCUSSION

The recent project of the study of pharmacological anti-inflammatory, analgesic and anti-spasmodic activity of *Punica granatum* was performed. Two extraction methanol and aqueous were analyzed for pharmacological potential. In Analgesic activity aqueous extract at dose 300 mg/kg showed noteworthy results as compared to standard drugs. The most efficient result is found in aqueous due to the presence of phenolic compounds. The carrageenan injection caused localized edema. The swelling amplified more and more to an extreme volume afterward the carrageenan injection. With the addition of methanol (300 mg/kg) had a major decrease of paw edema from1.00 to 0.60 in 3 hours. Similarly, inflammation property of some other plants was carried out by different researchers e.g. Scaphiumcnophorum, Turkish Myrtus species, Tridax procumbens Linn. The current conclusions agree with these researchers submitting the fact that communes has noteworthy anti-inflammatory influence (Gul *et al.*, 2019)

In anti-spasmodic activity of *Punica granatum* study was carried out. There was two friction methanol and aqueous was analyzed .In anti-spasmodic the methanol extract at dose 300mg/kg showed 78.33 % Inhibition is compared to standard drugs. The most efficient result is found in aqueous due to the presence of alkaloids. The Anti-spasmodic activities were done previously on many plants which have higher contents of flavonoids and essential oils which confirm our results. Flavone cirsimartin, which were obtained from *A.capillaris*, *A. xerophytica* and *Punica granatum* was responsible for the Antispasmolytic activity of isolated guinea pig ileum (Harborne *et al.*,2000) Four substance were extracted from the aerial parts of *Punica granatum* which possess antispasmolytic activity for smooth muscle relaxation (Puerta *et al.*, 2005). It was also found that oil from pomegranate has antispasmolytic substance has the ability to inhibit acetylcholine which cause contraction of isolated rat duodenum and the extract from leaves has also showed anti-diarrheal effect (Perfumi *et al*,. 2007).

IV. CONCLUSION

Overall the recent study showed that medicinal plants are best sources for treating many diseases of humans and animals. This study will also helpful to introduce drugs for infectious disease. The result of this analysis clearly showed that anti-inflammatory, Analgesic and anti-spasmodic activity increased with usage of plants. So it is quite clear that the value of *Punica granatam* locally increased for curing of many disorders. This study also suggest that *Punica granatam* is valuable sources of medication for many human disease

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V. References

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